REMARKS

Applicants thank the Examiner for the review of the instant application. Claims 1-5 are presented for examination. For the reasons stated below, Applicants respectfully traverse the rejection of the pending claims.

Rejection Under 35 U.S.C. §101

The PTO maintains its rejection of Claims 1-5 under 35 U.S.C. § 101 as lacking utility for the reasons set forth in the previous Office Action. The PTO asserts that while the differential expression of the PRO1335 mRNA in certain cancers "may provide utility and enablement for the PRO1335 DNA, it does not provide utility nor enablement for PRO1335 polypeptides or antibodies." *Office Action* at 3. While the PTO "agrees with the teachings of Alberts and Lewin that initiation of transcription is the most common point for a cell to regulate the gene expression, it is not the only means of regulating gene expression." *Id.* at 4 (emphasis added). The PTO cites several references to support its assertion that "the state of the art is such that polypeptide levels cannot be accurately predicted from mRNA levels." *Id.* at 8. The PTO concludes that "one skilled in the art would not consider it, more likely than not, that a small decrease in [mRNA] expression (no quantitative data provided) would correlate with significantly decreased polypeptide levels," and that "further research needs to be done to determine whether the small decrease in PRO1335 mRNA expression supports a role for the encoded polypeptide as a diagnostic marker in the cancerous tissue." *Id.* at 8.

Utility – Legal Standard

According to the Utility Examination Guidelines ("Utility Guidelines"), 66 Fed. Reg. 1092 (2001) an invention complies with the utility requirement of 35 U.S.C. § 101, if it has at least one asserted "specific, substantial, and credible utility" or a "well-established utility."

Under the Utility Guidelines, a utility is "specific" when it is particular to the subject matter claimed. For example, it is generally not enough to state that a nucleic acid is useful as a diagnostic tool without also identifying the condition that is to be diagnosed.

The requirement of "substantial utility" defines a "real world" use, and derives from the Supreme Court's holding in *Brenner v. Manson*, 383 U.S. 519, 534 (1966) stating that "The basic

quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility." In explaining the "substantial utility" standard, M.P.E.P. § 2107.01 cautions, however, that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, *any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient,* at least with regard to defining a 'substantial' utility." *M.P.E.P.* § 2107.01 (emphasis added).

The mere consideration that further experimentation might be performed to more fully develop the claimed subject matter does not support a finding of lack of utility. M.P.E.P. § 2107.01 III cites *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995) in stating that "Usefulness in patent law ... necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans." Further, "to violate § 101 the claimed device must be totally incapable of achieving a useful result." *Juicy Whip Inc. v. Orange Bang Inc.*, 51 U.S.P.Q.2d 1700 (Fed. Cir. 1999), *citing Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571 (Fed. Cir. 1992).

Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, set forth in M.P.E.P. § 2107 II(B)(1) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose ... and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Finally, in assessing the credibility of the asserted utility, the M.P.E.P. states that "to overcome the presumption of truth that an assertion of utility by the applicant enjoys" the PTO must establish that it is "more likely than not that one of ordinary skill in the art would doubt (i.e., 'question') the truth of the statement of utility." *M.P.E.P.* § 2107.02 III A. The M.P.E.P. cautions that:

> Rejections under 35 U.S.C. 101 have been **rarely sustained** by federal courts. Generally speaking, **in these rare cases**, the 35 U.S.C. 101 rejection was sustained [] because the **applicant ... asserted a utility that could only be true**

> **if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art.** *M.P.E.P.* § 2107.02 III B., citing *In re Gazave*, 379 F.2d 973, 978, 154 U.S.P.Q. 92, 96 (CCPA 1967) (underline emphasis in original, bold emphasis added).

Utility need NOT be Proved to a Statistical Certainty – a Reasonable Correlation between the Evidence and the Asserted Utility is Sufficient

An Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). *See also In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (1965); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977). Compliance with 35 U.S.C. § 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) cert. denied, 469 US 835 (1984). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. *M.P.E.P.* at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

In *Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996), the Court of Appeals for the Federal Circuit upheld a PTO decision that *in vitro* testing of a novel pharmaceutical compound was sufficient to establish practical utility, stating the following rule:

> [T]esting is often required to establish practical utility. But the test results **need not absolutely prove** that the compound is pharmacologically active. All that is required is that the tests be "*reasonably* indicative of the desired [pharmacological] response." In other words, there must be **a sufficient correlation** between the tests and an asserted pharmacological activity so as to convince those skilled in the art, **to a reasonable probability**, that the novel compound will exhibit the asserted pharmacological behavior." *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1564, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996) (internal citations omitted, bold emphasis added, italics in original).

While the *Fujikawa* case was in the context of utility for pharmaceutical compounds, the principals stated by the Court are applicable in the instant case where the asserted utility is for a therapeutic and diagnostic use – utility does not have to be established to an absolute certainty, rather, the evidence must convince a person of skill in the art "to a reasonable probability." In addition, the evidence need not be direct, so long as there is a "sufficient correlation" between the tests performed and the asserted utility.

The Court in *Fujikawa* relied in part on its decision in *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985). In *Cross*, the Appellant argued that basic *in vitro* tests conducted in cellular fractions did not establish a practical utility for the claimed compounds. Appellant argued that more sophisticated *in vitro* tests using intact cells, or *in vivo* tests, were necessary to establish a practical utility. The Court in *Cross* rejected this argument, instead favoring the argument of the Appellee:

> [*I*]*n vitro* results...are generally predictive of *in vivo* test results, i.e., there is a **reasonable correlation** therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. [Appellee] has not urged, and rightly so, that there is an invariable exact correlation between *in vitro* test results and *in vivo* test results. Rather, [Appellee's] position is that successful *in vitro* testing for a particular pharmacological activity establishes a **significant probability** that *in vivo* testing for this particular pharmacological activity will be successful. *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (emphasis added).

The *Cross* case is very similar to the present case. Like *in vitro* testing in the pharmaceutical industry, those of skill in the field of biotechnology rely on the reasonable

correlation that exists between gene expression and protein expression (see below). Were there no reasonable correlation between the two, the techniques that measure gene levels such as microarray analysis, differential display, and quantitative PCR would not be so widely used by those in the art. As in *Cross*, Applicants here do not argue that there is "an invariable exact correlation" between gene expression and protein expression. Instead, Applicants' position detailed below is that a measured change in gene expression in cancer cells establishes a "significant probability" that the expression of the encoded polypeptide in cancer will also be changed based on "a reasonable correlation therebetween."

Taken together, the legal standard for demonstrating utility is a relatively low hurdle. An Applicant need only provide evidence such that it is **more likely than not that a person of skill in the art would be convinced, to a reasonable probability, that the asserted utility is true**. The evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. The Applicant **does not need to provide evidence such that it establishes an asserted utility as a matter of statistical certainty.**

Even assuming that the PTO has met its initial burden to offer evidence that one of ordinary skill in the art would reasonably doubt the truth of the asserted utility, Applicants assert that they have met their burden of providing rebuttal evidence such that it is more likely than not those skilled in the art, to a reasonable probability, would believe that the claimed invention is useful as a diagnostic tool for cancer.

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

In an attempt to clarify Applicants' argument, Applicants offer a summary of their argument and the disputed issues involved. Applicants assert that the claimed antibodies have utility as diagnostic tools for cancer, particularly stomach, lung, rectal, and skin cancer. Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO1335 polypeptide is more highly expressed in normal stomach, lung, rectal, and skin tissue compared to stomach, lung, rectal and melanoma tumor, respectively;

2. Applicants assert that it is well-established in the art that a change in the level of mRNA for a particular protein, e.g. a decrease, generally leads to a corresponding change in the level of the encoded protein, e.g. a decrease;

3. Given Applicants' evidence that the level of mRNA for the PRO1335 polypeptide is decreased in stomach, lung, rectal and melanoma tumor, compared to normal tissue counterparts, it is likely that the PRO1335 polypeptide is likewise differentially expressed in stomach, lung, rectal and melanoma tumors;

4. The claimed antibodies to PRO1335 polypeptides therefore have utility as diagnostic tools for cancer.

Applicants understand the PTO to be making a single argument directed only to the second part of Applicants' asserted utility – while the PTO agrees "that initiation of transcription is the most common point for a cell to regulate the gene expression," the PTO asserts that "the state of the art is such that polypeptide levels cannot be accurately predicted from mRNA levels," and therefore, "further research needs to be done to determine whether the small decrease in PRO1335 mRNA expression supports a role for the encoded polypeptide as a diagnostic marker in the cancerous tissue." *Office Action* at 4, 8.

As detailed below, Applicants submit that the PTO has failed to demonstrate that this is one of the "rare cases" where the applicants have "asserted a utility that could only be true if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art." *M.P.E.P.* § 2107.02 III B. Applicants submit that the references cited by the PTO are either irrelevant, not contrary to Applicants' arguments, or actually offer support for Applicants' position. Even if the PTO has met its initial burden, Applicants have submitted enough rebuttal evidence such that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated above, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Applicants have established that the Gene Encoding the PRO1335 Polypeptide is Differentially Expressed in Certain Cancers compared to Normal Tissue

Applicants note that in the closely related application Serial No. 10/063,709, directed to nucleic acids related to SEQ ID NO:73 which encodes the PRO1335 polypeptide, the PTO has issued an Notice of Allowability, acknowledging that the nucleic acids have utility and are enabled. *See Notice of Allowability dated 12/13/2005.* In that case, the exact same data from Example 18 was relied on for utility of the claimed nucleic acids as diagnostic tools for stomach, lung, rectal, and melanoma tumors. In response to Applicants' arguments that the differential expression of the PRO1335 mRNA makes the claimed nucleic acids useful as diagnostic tools, the PTO stated that "[t]he rejections under 35 U.S.C. § 101 and §112, first paragraph, are withdrawn in view of Applicant's persuasive arguments." *Id.* at 2. Therefore, Applicants submit that any questions regarding the significance or reliability of the exact same data in the instant case is moot in light of this statement.

Applicants submit that the evidence reported in Example 18, combined with the first Grimaldi Declaration previously submitted, establish that there is at least a two-fold difference in PRO1335 cDNA between stomach, lung, rectal, and melanoma tumor tissue compared to their normal tissue counterparts. Therefore, it follows that expression levels of the PRO1335 gene can be used to distinguish stomach, lung, rectal and melanoma tumor tissue from their normal tissue counterparts. The PTO has not offered any significant arguments or evidence to the contrary, and the PTO has accepted this same assertion in the related application, Serial No. 10/063,709, directed to nucleic acids related to SEQ ID NO:73 which encodes the PRO1335 polypeptide. *See Notice of Allowability dated 12/13/2005* at 2.

As Applicants explain below, it is more likely than not that the PRO1335 polypeptide and antibody can also be used to distinguish stomach, lung, rectal, and skin tumor tissue from their normal tissue counterparts.

Applicants have established that the Accepted Understanding in the Art is that there is a Positive Correlation between Changes in mRNA Levels and Changes in the Level of Expression of the Encoded Protein

Applicants next turn to the second portion of their argument in support of their asserted utility – that it is well-established in the art that a change in the level of mRNA for a particular protein, generally leads to a corresponding change in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO1335 polypeptide in stomach, lung, rectal and melanoma tumor, it is likely that the PRO1335 polypeptide is likewise differentially expressed; and antibodies to proteins differentially expressed in certain tumors have utility as diagnostic tools.

The PTO's cited references are not contrary to Applicants' asserted utility

In response to Applicants' assertion, the PTO cites Hu *et al.* (J. Proteome Res. 2003; 2(4):405-12), LaBaer (Nature Biotech. 2003; 21:976-977), Haynes *et al.* (Electrophoresis 1998; 19(11):1862-71), Gygi *et al.* (Mol. and Cell. Bio., Mar. 1999; 1720-1730), Chen *et al.* (Mol. and Cell. Proteomics 2002; 1:304-313), Lichtinghagen *et al.* (European Urology 2002; 42:398-406), Lian *et al.* (Blood 2001; 98:513-524) and Fessler *et al.* (J. Biol. Chem. 2002; 277:31291-31302) as support for its argument that "in organisms ranging from yeast to human, changes in mRNA levels are not predictive of changes in the encoded polypeptide levels, especially in cancerous cells." *Office Action* at 5. For the reasons discussed below, Applicants submit that the references cited by the PTO are either irrelevant, not contrary to Applicants' arguments, or actually offer support for Applicants' position.

a. Hu et al. and LaBaer

Applicants turn first to the PTO's arguments based on Hu *et al.* and LaBaer. In Hu, the researchers used an automated literature-mining tool to summarize and estimate the relative strengths of all human gene-disease relationships published on Medline. They then generated a microarray expression dataset comparing breast cancer and normal breast tissue. Using their data-mining tool, they looked for a correlation between the strength of the literature association between the gene and breast cancer, and the magnitude of the difference in expression level.

They report that for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. *See* Hu at 411. However, among genes with a 10-fold or more change in expression level, there was a strong correlation between expression level and a published role in the disease. *Id.* at 412. Importantly, Hu reports that the observed correlation was only found among estrogen receptor-positive tumors, not ER-negative tumors. *Id.*

The general findings of Hu are not surprising – one would expect that genes with the greatest change in expression in a disease would be the first targets of research, and therefore have the strongest known relationship to the disease as measured by the number of publications reporting a connection with the disease. The correlation reported in Hu only indicates that the greater the change in expression level, the more likely it is that there is a published or known role for the gene in the disease, as found by their automated literature-mining software. Thus, Hu's results merely reflect a bias in the literature toward studying the most prominent targets, and reflect nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker.

Hu acknowledges the shortcomings of this method in explaining the disparity in Hu's findings for ER-negative versus ER-positive tumors: Hu attributes the "bias in the literature" toward the more prevalent ER-positive tumors as the explanation for the lack of any correlation between number of publications and gene expression levels in less-prevalent (and, therefore, less studied) ER-negative tumors. *Id.* Because of this intrinsic bias, Hu's methodology is unlikely to ever note a correlation of a disease with less differentially-expressed genes and their corresponding proteins, regardless of whether or not an actual relationship between the disease and less differentially-expressed genes exists. Accordingly, Hu's methodology yields results that provide little or no information regarding biological significance of genes with less than 5-fold expression change in disease.

More importantly, Hu did not look for a correlation between changes in mRNA and changes in protein levels, and therefore is not contrary to Applicants' assertion that there is a correlation between the two. Applicants are not relying on any "role" that PRO1335 has in cancer for their asserted utility. Instead, Applicants are relying on the differential expression of PRO1335 in certain tumors compared to their normal tissue counterparts. Nowhere in Hu does it

say that a lack of correlation in their study means that genes with a less than five-fold change in level of expression in cancer cannot serve as a molecular marker of cancer.

The PTO also relies on the publication by LaBaer to broaden its interpretation of Hu. *Office Action* at 5. The PTO points to a statement in LaBaer that:

> In the accelerating quest for disease biomarkers, use of high-throughput technologies, such as DNA microarrays and proteomics experiments, has produced vast datasets identifying thousands of genes whose expression patterns differ in diseased versus normal samples. Although many of these differences may reach statistical significance, they are not always biologically meaningful. For example, reports of mRNA or protein changes of as little as two-fold are not uncommon, and although some changes of this magnitude turn out to be important, most are attributable to disease-independent differences between samples. Office Action at 14 (emphasis added). *LaBaer* at 976.

LaBaer is an unreviewed letter to the editor by an author of the Hu *et al.* article describing the automated literature searching tool used in the Hu *et al.* reference discussed above. LaBaer provides no further evidence than that provided in Hu, and provides no evidence whatsoever to support the conclusion that the results of Hu are applicable to the diagnostic utility of differentially expressed genes. Importantly, like the Hu reference, LaBaer does not consider or offer any discussion of whether there is a correlation between changes in mRNA levels and changes in the level of the encoded protein.

In addition, it is important to note that Applicants' are not relying on microarray data as discussed in Hu and LaBaer. Instead, they are relying on a more accurate and reliable method of assessing changes in mRNA level, namely quantitative PCR analysis. In a recent study by Kuo *et al.*, (Proteomics 5(4):894-906 (2005)), the authors used microarray analysis combined with proteomic analysis using two-dimensional gel electrophoresis to examine changes in gene expression in leukemia cell lines, just as discussed in LaBaer. The authors report that "[c]omparison of microarray and proteomic expression profiles showed poor correlation. Use of more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, on several genes and proteins, nonetheless, confirmed that there is indeed good correlation between mRNA and protein expression." Kuo *et al.* at Abstract (emphasis added) (attached as Exhibit 1). Thus, even if accurate, Hu and LaBaer's statements regarding microarray studies are not relevant to the instant application which does not rely on microarray data.

Moreover, LaBaer is silent regarding the reliability of pooled samples, and whether or not differential expression in pooled samples are susceptible to disease-independent differences between samples. LaBaer's conclusions regarding disease-independent differences between samples are not applicable in the instant case where normal human tissue and corresponding human tumor tissue samples were used. Accordingly, LaBaer suffers from the same defects discussed above with respect to Hu *et al.*

In conclusion, nothing in either LaBaer or Hu addresses Applicants' assertion that there is a correlation between changes in mRNA level and changes in the level of the encoded polypeptide. As such, they are not relevant to establishing whether the claimed antibodies have utility based on the differential expression of the PRO1335 mRNA in stomach, lung, rectal, and melanoma tumors.

b. Haynes et al. and Gygi et al.

The PTO relies on Haynes *et al.* and Gygi *et al.* to support its assertion that "transcript levels do not necessarily correlate with protein levels in normal tissue." *Office Action* at 6-7.

Haynes studied whether there is a correlation between the level of mRNA expression and the level of protein expression for 80 selected genes from yeast. The genes were selected because they constituted a relatively homogeneous group with respect to predicted half-life and expression level of the protein products. *See Haynes* at 1863. Haynes did not examine whether a change in transcript level for a particular gene led to a change in the level of expression of the corresponding protein. Instead, Haynes determined whether the steady-state transcript level correlated with the steady-state level of the corresponding protein based on an analysis of 80 different genes.

The PTO focuses on the portion of Haynes where the authors reported that for some of the studied genes with equivalent mRNA levels, there were differences in corresponding protein expression, including some that varied by more than 50-fold. *Office Action* at 6. Similarly, Haynes reports that different proteins with similar expression levels were maintained by transcript levels that varied by as much as 40-fold. Thus, Haynes showed that in yeast, similar steady-state mRNA levels for different genes did not universally result in equivalent steady-state protein levels for the different gene products, and that similar steady-state protein levels for

different gene products did not universally result from equivalent steady-state mRNA levels for the different genes. These results are expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, based on these results, Haynes concluded that protein levels cannot always be accurately predicted from the level of the corresponding steady-state mRNA transcript when looking at the level of transcripts across different genes.

Importantly, Haynes did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing or decreasing the level of mRNA for a particular gene leads to a corresponding increase or decrease for the encoded protein. Haynes did not study this issue and says absolutely nothing about it. One cannot look at the steady-state level of mRNA across several different genes to investigate whether a change in the level of mRNA a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes is not inconsistent with or contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

The PTO also relies on Gygi *et al.*, a study on which the Haynes references is based. *Office Action* at 7. Like Haynes, the Gygi reference looked at steady-state levels of mRNA across different genes, not changes in mRNA levels for a single gene. Thus, when Gygi *et al.* state that "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data," the authors are referring to correlations between steady-state levels of mRNA and protein across different genes, not a correlation between a change in mRNA level and a change in protein level for the same gene and corresponding protein. Therefore, for the same reasons that Haynes is not relevant to Applicants' asserted utility, Gygi likewise offers no support for the PTO's rejection of Applicants' asserted utility.

c. Chen et al.

The PTO also cites Chen *et al.* for support for the assertion that polypeptide levels cannot be accurately predicted from mRNA levels. *See Office Action* at 5-6. In Chen, the authors

examined the relationship between mRNA levels and protein levels in 76 lung adenocarcinomas and 9 non-tumor lung samples.

Like Haynes and Gygi, Chen examined the global relationship between mRNA and the corresponding protein abundance by calculating the average mRNA and protein level of all the samples for each gene or protein, and then looked for a correlation across the different genes. Thus, Chen's statement repeated by the PTO that "it is not possible to predict overall protein expression levels based on average mRNA abundance in lung cancer samples" is referring to a correlation between steady-state mRNA levels and protein levels across different genes. As discussed above with respect to Haynes and Gygi, this measurement of a correlation across different genes is not relevant to Applicants' asserted utility which relies in part on a correlation between changes in a gene's expression level and changes in the level of the encoded protein.

In addition to looking at global correlations across genes, Chen also looked at the level of mRNA of 98 individual genes and their corresponding proteins across the samples. Chen reports that 21.4% (21 of 98) of the genes showed a statistically significant correlation between protein and mRNA expression.

Chen provides scant evidence to counter Applicants' asserted utility for the claimed antibodies because when examined closely, portions of Chen support Applicants' assertions, and the remaining portions provide little insight into the relationship between changes in mRNA levels and corresponding protein levels for mRNA that is differentially expressed in tumor cells relative to normal cells. Rather than looking for mRNAs which were differentially expressed, Chen merely selected proteins whose identity could be determined regardless of any changes in expression level. *Chen* at 306, right column. Importantly, it is not known if there was any substantial difference in mRNA levels for the various genes across samples – in short, with the exception of the genes in Figures 2A-2C, it is not known if the genes examined were differentially expressed. Also of significance for Applicants' asserted utility is the fact that Chen did not attempt to examine any differential expression between the cancerous lung samples and the non-cancerous lung samples – Chen did not distinguish between cancer and normal samples in their analysis.

Applicants have asserted that changes in mRNA levels, particularly those which are two-fold or greater, will correspond with measurable changes in polypeptide expression. The only

data showing a change in mRNA level in Chen support Applicants' assertion. In Figures 2A-2C, Chen plots mRNA value vs. protein value for three genes. In these figures, a wide range of mRNA expression levels were observed (approximately seven- to eight-fold), and a correlation between mRNA and protein levels was observed for all three mRNA/protein pairs. This supports Applicants' asserted correlation between changes in mRNA levels which are two-fold or greater and changes in polypeptide expression.

The PTO relies on the fact that Chen also reports a lack of correlation for some mRNA/protein pairs to support its assertion that polypeptide levels cannot be accurately predicted from mRNA levels. However, the lack of correlation reported by Chen could be a result of a lack of substantial changes in mRNA level. This can be understood by again turning to Figures 2A-2C. As noted above, where a wide range of mRNA expression levels are seen, a correlation between mRNA and protein levels was observed. However, if one examines the data points within a small range of mRNA levels for these same genes, e.g. 500-600 or 5000-6000 in Figs. 2A-2C, it is clear that a correlation would not be detected for the data within this range. This does not mean that a correlation between changes in mRNA and protein does not exist for these genes, as is evident when larger changes in mRNA expression are included in the analysis. Instead, this indicates that for relatively small changes in mRNA, any correlation is masked by imprecision in the measurements.

Chen's experiment compared mRNA levels vs. protein levels across samples without selecting mRNA that showed a difference in expression level. And unlike Applicants, Chen did not examine differences in mRNA between tumor and normal tissue. Since almost all samples tested by Chen were from the same type of tissue, few substantial variations in the level of mRNA or protein for a particular gene across the samples tested would be expected. Instead, it would be expected that most genes examined by Chen would have similar mRNA or protein levels across the samples. Figures 2A-2C of Chen demonstrate that the methods utilized by Chen cannot detect correlations between mRNA and protein levels when only small differences in mRNA expression are observed, but a correlation is detected when larger differences in mRNA expression are observed.

Accordingly, the only data reported by Chen which shows substantial changes in the expression of mRNA, Figures 2A-C, confirms Applicants' assertion that substantial changes in

mRNA levels (e.g., 2-fold or greater) will correspond to substantial changes in polypeptide expression. Further, this data also explains the lack of observed correlation between mRNA levels and protein levels for other genes reported by Chen. Thus, even given Chen's inability to detect a correlation between mRNA and protein in some genes, Chen's results do not refute Applicants' position.

Instead, Chen supports Applicants' position that a significant correlation between mRNA and protein levels exists for changes in mRNA levels that are 2-fold or greater. In further support of Applicants' position, Chen cites Celis *et al.* (FEBS Lett. 2000; 480:2-16) stating that the authors "found a good correlation between transcript and protein levels among 40 well resolved, abundant proteins using a proteomic and microarray study of bladder cancer." *Chen* at 311, first column (emphasis added). As mentioned above, the lack of a correlation across genes is not relevant to Applicants' asserted utility, and therefore Chen's discussion of this issue and citation of Anderson and Seilhamer (Electrophoresis 1997; 18:533-37) and Gygi *et al.* (Mol. Cell. Bio. 1999; 19:1720-30) offer no support for the PTO's position.

In conclusion, for the reasons discussed above with respect to Haynes and Gygi, portions of Chen are simply irrelevant to the discussion of whether or not the changes in mRNA lead to corresponding changes in the level of encoded protein because Chen was looking at mRNA and protein levels across different genes. The remainder of Chen is offers little or no support for the PTO's position since Chen did not examine genes where a change in mRNA level was known to occur. In the three cases where it is certain that changes in mRNA level did occur, there is a correlation between the changes in the level of mRNA and the changes in the level of the corresponding protein.

d. Lichtinghagen et al.

In addition to Chen *et al.*, the PTO also cites Lichtinghagen *et al.*, stating that the reference shows a similar lack of correlation in matrix metalloproteinases (MMPs 2 and 9) and the tissue inhibitor of metalloproteinase 1 (TIMP-1) in human prostate cancer. The PTO relies on the statement from Lichtinghagen that "[c]omparison of mRNA and protein expression of MMP-2, MMP-9 and TIMP-1, respectively, did not show any significant relationships illustrating the necessity to study these components at both molecular levels." *Office Action* at 6.

Lichtinghagen examined the level of MMP-2, MMP-9 and TIMP-1 in cancerous and non-cancerous parts of 17 human prostate samples at both the mRNA and protein level. The level of mRNA was determined using RT-PCR, and the level of protein was determined using quantitative zymography and ELISA. Lichtinghagen reports that comparing non-cancerous to cancerous tissue, mRNA levels were decreased for MMP-2, and unchanged for MMP-9 and TIMP-1. *See Lichtinghagen* at Abstract. In contrast, looking at the protein level, MMP-2 levels were unchanged, while MMP-9 expression was higher and TIMP-1 levels were lower. *Id.* Thus, Lichtinghagen reports that there was no correlation between mRNA levels and protein levels. *Id.*

First, it is important to note that of the three genes examined, only one (MMP-2) showed any change in mRNA expression levels between cancerous and non-cancerous tissues. While statistically significant, the change was small (approximately 33% decrease), far less than a two-fold change. It is therefore not surprising that the authors did not see a measurable change in the amount of MMP-2 protein.

For MMP-9 and TIMP-1, the authors report that there was no change in the level of mRNA, but there was a change in protein level. This apparent lack of correlation between mRNA and protein levels is not contrary to Applicants' assertion that a change in mRNA level generally leads to a change in protein level. Applicants are not attempting to predict the level of mRNA based on changes in protein level, and Applicants have not asserted that the only means for changing the level of protein is to change the amount of the encoding mRNA. Therefore a change in protein without a change in mRNA is not contrary to Applicants' assertions.

Second, the authors in Lichtinghagen note that in another study, researchers found a direct correlation between mRNA levels and protein levels for MMP-2 in prostate cancer. *See Lichtinghagen* at 403, col. 2, *citing* Stearns and Wang (Cancer Res. 1993; 53(4):878-83). In the Stearns and Wang reference cited in Lichtinghagen, the authors report differences in MMP-2 mRNA levels between cancerous, benign and normal stromal tissue from human prostate. The authors state that "[e]nzyme-linked immunosorbent assays demonstrated that the amounts of type IV collangenase protein [MMP-2 protein] correlated directly with the mRNA levels in the tumor tissue." *Stearns and Wang* at Abstract (abstract attached hereto as Exhibit 2). Therefore, contrary to the results reported in Lichtinghagen, at least one other study reports a good correlation between changes in mRNA and protein levels for MMP-2 in prostate cancer.

In conclusion, Lichtinghagen is not contrary to Applicants' assertion that generally, a change in mRNA level leads to a corresponding change in protein level. Lichtinghagen reported a single gene where an apparent change in mRNA did not result in a corresponding change in the level of protein. However, the change in mRNA level was very small, and other researchers have reported a direct correlation between mRNA levels and protein levels for the same gene in human prostate samples. The two other genes examined by Lichtinghagen did not show a change in mRNA level, and therefore say nothing about Applicants' assertion.

e. Lian et al.

The PTO cites Lian *et al.* for the statement that there is a poor correlation between mRNA expression and protein abundance in mouse cells, and therefore it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels. *Office Action* at 7.

In Lian, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. *Lian* at Abstract. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization, and protein levels were qualitatively assessed following 2-dimensional gel electrophoresis. *Id.* at Abstract, Table 6.

Lian *et al.* used DD and array hybridization to examine the expression of genes 0, 24, 48 and 72 hours after treatment with retinoic acid. *Id.* at 515, col. 1, ¶ 2. Using this information, the authors constructed a database of mRNA level changes during differentiation of the cell line. *Id.* at 518, col. 2, ¶ 2. Lian *et al.* also examined protein expression at 0 and 72 hours after retinoic acid treatment. Lian reports that they were able to identify 28 proteins which they considered differentially expressed. *Id.* at 521, Fig. 5. Of those 28, only 18 had corresponding gene expression information in the database, and only 13 had measurable levels of mRNA expression. *Id.* at 521, Table 6. The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels. *Id.* at 512, col. 1. The authors note that "[n]one of these was on the list of genes that were

differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)." *Id.* at 512, bridge paragraph (emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in 2DE, there was poor correlation between changes in mRNA levels and estimated protein levels." *Id.* at 522, col. 2, ¶ 2.

These results are not contrary to Applicants' assertion. Applicants emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: m elanoma X-actin, which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between a change in mRNA level and a corresponding change in protein level. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. *Id.* at 521, Table 6. Thus, there is little basis for the authors' conclusion relied on by the PTO that "it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels (as estimated from 2DE)." *Office Action* at 7 (emphasis added).

f. Fessler et al.

The PTO also cites a publication by Fessler *et al.* Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. Fessler lists in Table VIII a comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of the 13 up-regulated proteins, a change in mRNA levels is reported for only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Of the 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Regarding the remainder of the proteins listed in Table VIII, in 6 instances, protein levels changed while mRNA levels were unchanged. This evidence has no relevance to Applicants' assertion that changes in mRNA levels lead to corresponding changes in protein levels, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that changes in mRNA levels causes corresponding changes in protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased, decreased or remained unchanged in PMN upon contact with LPS. Nothing in these results by Fessler suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

The PTO points to Fessler's statement regarding Table VIII that "a poor concordance between mRNA transcript and protein expression changes." *Office Action* at 7. As is clear from the above discussion, this statement does not relate to a lack of correlation between a change in mRNA levels leading to a change in protein levels, because in 5 of 6 such instances, changes in mRNA and protein levels correlated well. Instead, this statement relates to observations in which protein levels changed when mRNA was either unchanged or "absent." As such, this statement is an observation that in addition to transcriptional activity, LPS also has post-transcriptional and possibly post-translational activity that affect protein levels, an observation which is not contrary to Applicants' assertions. Accordingly, Fessler's results are consistent with Applicants' assertion that a change in mRNA level of for a particular protein generally leads to a corresponding change in the level of the encoded protein, since 5 of 6 genes demonstrated such a correlation.

g. Conclusion – the PTO's cited references do not offer significant evidence to question Applicants' asserted utility

The PTO's rejection of Applicants' asserted utility is based on a rejection of Applicants' conclusion that because the PRO1335 mRNA expression is decreased in stomach, lung, rectal and melanoma tumors compared to normal tissue, the PRO1335 polypeptide expression will be decreased as well. This conclusion is not based on the assertion that steady-state mRNA levels are predictive of protein levels when comparing different genes, or that one can determine the level of mRNA based on changes in protein level. It is based on Applicants' assertion that changes in mRNA level generally result in corresponding changes in the level of the encoded protein. In rejecting this conclusion, the PTO has cited references by Hu *et al.*, LaBaer, Haynes *et al.*, Gygi *et al.*, Chen *et al.*, Lichtinghagen *et al.*, Lian *et al.*, and Fessler *et al.*

As explained above, Hu and LaBaer do not even discuss whether there is a correlation between mRNA and protein levels. While Haynes and Gygi address the relationship between mRNA and protein levels generally, their studies where limited to investigation of steady-state mRNA levels and correlations across different genes – a relationship which is irrelevant to Applicants' conclusion.

While a portion of Chen is more relevant in that the authors were not looking at steady-state levels of mRNA across different genes, the authors made no attempt to look at mRNAs

which were differentially expressed. Therefore, it is not known if the observed lack of correlation in Chen is contrary to Applicants' assertion, or merely the result of examining genes that did not exhibit substantial changes in mRNA level. The data in Chen show only 3 genes where it is known that mRNA levels were substantially changed, and in all three cases there was a correlation between mRNA and protein levels.

As pointed out above, a lack of correlation between mRNA levels and protein levels when mRNA levels are unchanged or not detectable is not contrary to Applicants' assertion – Applicants are not attempting to predict mRNA levels based on changes in protein level. It is for this reason that Lichtinghagen does not offer any support for the PTO's position. In Lichtinghagen, the authors examined three genes, two of which showed a change in protein level but no change in mRNA levels – results which are irrelevant to Applicants' assertion. Only one gene showed a change in mRNA level, a small ~33% decrease. In that case, the authors observed no change in the corresponding protein level. However, other researchers have reported a good correlation between mRNA and protein levels for the same gene in the same kind of tissue. Thus, Lichtinghagen does not offer any support for the PTO's position. Similarly, in Lian only two genes for which both mRNA and protein levels were studied showed differential mRNA expression. In one case protein levels showed a corresponding change, while in another they apparently did not. Finally, in Fessler, only 6 mRNAs showed a change in the level of expression. Fessler reports that for 5 of the 6, protein levels showed a corresponding change.

When taken together, there are 12 examples of substantial changes in mRNA levels reported in the PTO's cited references (3 in Chen, 1 in Lichtinghagen, 2 in Lian, and 6 in Fessler). In the remainder of the examples from the cited references, mRNA levels were not changed, were absent or not detectable, or it is unknown if mRNA levels changed. For the 12 genes where a substantial change in mRNA was reported, the authors found a correlation between changes in mRNA level and the level of the corresponding protein in 9 of the 12 examples. Thus, taken as a whole, the references cited by the PTO do not support the PTO's rejection of Applicants' assertion that more often than not, there is a correlation between changes in mRNA level and changes in the level of the corresponding protein. If anything, the cited references support Applicants' position.

Applicants' previously submitted supporting declarations and references

In support of the assertion that changes in mRNA are positively correlated to changes in protein levels, Applicants previously submitted a copy of a second Declaration by J. Christopher Grimaldi, an expert in the field of cancer biology. As stated in paragraph 5 of the declaration, "Those who work in this field are well aware that in the vast majority of cases, when a gene is over-expressed...the gene product or polypeptide will also be over-expressed.... This same principal applies to gene under-expression." Further, "the detection of increased mRNA expression is expected to result in increased polypeptide expression, and the detection of decreased mRNA expression is expected to result in decreased polypeptide expression. The detection of increased or decreased polypeptide expression can be used for cancer diagnosis and treatment." The references cited in the declaration and submitted herewith support this statement.

Applicants also previously submitted a copy of the declaration of Paul Polakis, Ph.D., an expert in the field of cancer biology. As stated in paragraph 6 of his declaration:

> Based on my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above [showing a positive correlation between mRNA levels and encoded protein levels in the vast majority of cases] and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, *it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein.* (Emphasis added).

Dr. Polakis acknowledges that there are published cases where such a correlation does not exist, but states that it is his opinion, based on over 20 years of scientific research, that "such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein." *Polakis Declaration*, at ¶ 6.

The statements of Grimaldi and Polakis are supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (previously submitted, herein after Cell 3rd) and (4th ed. 2002) (previously submitted, herein after Cell 4th)). Figure 9-2 of Cell 3rd shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is transcriptional control. Cell 3rd provides

that "[f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Cell 3rd at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Cell 3rd at 453 (emphasis added). Thus, as established in Cell 3rd, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Cell 4th, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA*." Cell 4th at 302 (emphasis added). Similarly, Figure 6-90 on page 364 of Cell 4th illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Cell 4th at 364 (emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (previously submitted) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004 (previously submitted). Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" *Zhigang* at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate

cancer, and that the increased protein level of PSCA was resulted from the upregulated transcription of its mRNA." *Zhigang* at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." *Id.* at 7

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002), previously submitted, states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the declarations of Grimaldi and Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

Applicants' additional supporting references

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a comprehensive study by Orntoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) (previously submitted with IDS, attached hereto as Exhibit 3), the authors examined gene amplification, mRNA expression level, and protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. The alternations in mRNA and

protein included both increases and decreases. *Id.* at 43, Table II. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (abstract attached as Exhibit 4) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and nonneoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (abstract attached as Exhibit 5) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 6) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract.

The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 7) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (abstract attached as Exhibit 8) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level.

Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 9). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 10) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 11), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals

for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, e.g. a decrease.

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 12) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, e.g. an increase, generally leads to a corresponding change in the level of protein expression, e.g. an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an additional 70 references (abstracts attached as Exhibit 13) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer indirect support of Applicants' asserted utility. As discussed above, Applicants have challenged the relevance of references such as Haynes *et al.*, Gygi *et al.* and Chen *et al.*, which do not attempt to examine the correlation between a change in mRNA level and a change in the level of the corresponding protein level. Because the PTO continues to rely on these references, Applicants are submitting references which report results that are contrary to the PTO's cited references and offer indirect support for Applicants' asserted utility.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (abstract attached as Exhibit 14) the authors conducted a study of mRNA and protein expression in yeast which was nearly identical to the one conducted by Gygi *et al.* Contrary to the results of the earlier study by Gygi, Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 15) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 16) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 17) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 18) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 19) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 20) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin

D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 21) which also support Applicants' assertion in that they report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

In summary, Applicants submit herewith a total of 113 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 22). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO1335 mRNA is more highly expressed in normal stomach, lung, rectal and skin tissue compared to stomach, lung, rectal and melanoma tumor, respectively, the PRO1335 polypeptide will likewise be differentially expressed in stomach, lung, rectal and melanoma tumor. This differential expression of the PRO1335 polypeptide makes the claimed antibodies useful as diagnostic tools for cancer, particularly stomach, lung, rectal and melanoma cancer.

The Arguments made by the PTO are Not Sufficient to satisfy the PTO's Initial Burden of Offering Evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility"

As stated above, an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

Applicants remind the PTO that the M.P.E.P. cautions that rejections for lack of utility are rarely sustained by federal courts, and that generally speaking, a utility rejection was sustained because the applicant asserted a utility "that could **only be true if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art.**" M.P.E.P. § 2107.02 III B., citing *In re Gazave*, 379 F.2d 973, 978, 154 U.S.P.Q. 92, 96 (CCPA 1967) (underline emphasis in original, bold emphasis added). Rather than being wholly inconsistent with contemporary knowledge in the art, Applicants' asserted utility is squarely within the teaching of leading textbooks in the field, and is supported by references and the declarations of skilled experts.

Applicants' asserted utility is based on the assertion that changes in mRNA level generally result in corresponding changes in the level of the encoded protein. In rejecting this conclusion, the PTO has cited references by Hu *et al.*, LaBaer, Haynes *et al.*, Gygi *et al.*, Chen *et al.*, Lichtinghagen *et al.*, Lian *et al.*, and Fessler *et al.*

As explained above, these references are largely irrelevant to determining whether Applicants' asserted utility is more likely than not true. When taken together, there are 12 examples of substantial changes in mRNA levels reported in the PTO's cited references, and in 9 of the 12 examples the authors found a correlation between changes in mRNA level and changes in the level of the corresponding protein. Thus, taken as a whole, the references cited by the PTO do not support the PTO's rejection of Applicants' asserted utility.

Given the lack of support for the PTO's position, Applicants submit that the PTO has not met its initial burden of overcoming the presumption that the asserted utility is sufficient to satisfy the utility requirement. And even if the PTO has met that burden, the Applicants' supporting rebuttal evidence, including two uncontested expert declarations, excerpts from three textbooks, and over 115 scientific articles, is more than sufficient to establish that one of skill in the art would be more likely than not to believe that the claimed antibodies can be used as diagnostic tools for cancer, particularly stomach, lung, rectal and melanoma cancer.

Specific Utility

The Asserted Substantial Utilities are Specific to the Claimed Antibodies

Applicants next address the PTO's assertion that the asserted utilities are not specific to the claimed antibodies to PRO1335 polypeptide. Applicants respectfully disagree.

Specific utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." M.P.E.P. § 2107.01 I. Applicants submit that the evidence of differential expression of the PRO1335 gene and polypeptide in certain types of tumor cells, along with the declarations and references discussed above, provide a specific utility for the claimed antibodies.

As discussed above, there are significant data which show that the gene for the PRO1335 polypeptide is more highly expressed in normal stomach, lung, rectal, and skin tissue compared to stomach, lung, rectal and melanoma tumor, respectively. These data are strong evidence that

the PRO1335 gene and polypeptide are associated with stomach, lung, rectal and melanoma tumors. Thus, contrary to the assertions of the PTO, Applicants submit that they have provided evidence associating the PRO1335 gene and polypeptide with a specific disease. The asserted utility as a diagnostic tool for cancer, particularly stomach, lung, rectal and melanoma tumor, is a specific utility – it is not a general utility that would apply to the broad class of antibodies.

Conclusion

The PTO has asserted a single argument to support its rejection of Applicants' asserted utility: "the state of the art is such that polypeptide levels cannot be accurately predicted from mRNA levels." *Office Action* at 8. Applicants have addressed each of the PTO's supporting references and shown that they are either irrelevant, or taken as a whole, actually support Applicants' assertion that a change in mRNA level leads to a corresponding change in the level of the encoded protein. In addition, Applicants have submitted expert declarations, textbook excerpts, and over 115 scientific publications which support Applicants' asserted utility.

Given the totality of the evidence provided, Applicants submit that they have established a substantial, specific, and credible utility for the claimed antibodies as diagnostic tools. According to the PTO Utility Examination Guidelines (2001), irrefutable proof of a claimed utility is not required. Rather, a specific, substantial, and credible utility requires only a "reasonable" confirmation of a real world context of use. Applicants remind the PTO that:

> A small degree of utility is sufficient . . . The claimed invention must only be capable of performing **some** beneficial function . . . An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely... A commercially successful product is not required... Nor is it essential that the invention accomplish all its intended functions... or operate under all conditions... partial success being sufficient to demonstrate patentable utility... In short, **the defense of non-utility cannot be sustained without proof of total incapacity**. If an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate. M.P.E.P. at 2107.01 (underline emphasis in original, bold emphasis added, citations omitted).

Applicants submit that they have established that it is more likely than not that one of skill in the art would reasonably accept the utility for the claimed antibodies relating to PRO1335

set forth in the specification. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, first paragraph – Enablement

The PTO also rejects Claims 1-5 under 35 U.S.C. § 112, first paragraph, as lacking enablement because the claimed invention is not supported by either a specific or substantial asserted utility or a well-established utility. *Office Action* at 11.

Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed antibodies. Applicants respectfully request that the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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